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SOME RESULTS OF A BACTERIOLOGICAL EXAMINATION OF THE PIPETTES AND COLLYRIA TAKEN FROM A TREATMENT CASE USED IN OPHTHALMIC PRACTICE, WITH THE EFFECTS OF INOCULATIONS.

A Preliminary Communication.

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The facility with which various fungi grow in many of the lotions commonly used in the treatment of ocular diseases, and particularly the proneness of the solutions of the alkaloids—cocaine, atropine, eserine—to accept this kind of contamination, has for a long time influenced the direction of much attention to the best methods of sterilization under these circumstances. True, these fungi and germs are not always, or not usually, pathogenic in the ordinary acceptation of the word; but the cases of wound infection—for example, after cataract extraction—from the use of unclean solutions, the microbic origin of toxic conjunctivitis (atropine, eserine and cocaine conjunctivitis), and the probability, as Philippson[1] declares, that certain ulcers of the cornea owe their origin to infected atro-

[1] Ueber Ulcus corn. serp., durch Einträufelung septischer Atropinlösungen hervorgerufen. Hosp. Tid. R. iii., 1885; quoted by Franke, Archiv f. Ophthal., Bd. xxxvii. Abth. ii. 73.

pine drops, furnish grounds for the exercise of every proper precaution to secure clean fluids, bottles and pipettes.

The germs, as Franke has shown, may be present in the bottles and pipettes, or as Davidson[1] has demonstrated, in the distilled water (micrococcus aquatalis, etc.); again, they may come from the surrounding air; or, finally—and this, as Franke insists, presents the greatest difficulty to overcome—they may be introduced into the lotions by the surgeon himself when, for example, the end of the pipette has accidentally touched the conjunctiva or the eyelashes, and thus carried into the bottle some of the micro-organisms so commonly present in these structures.

Franke, to whose research we have referred, gives a very interesting *résumé* of the various procedures to secure sterilization under these circumstances. From the time that Kroemer added to solutions of the alkaloids salicylic acid 1 : 400, boric acid 4 : 100, and carbolic acid 1 : 1000, which even in this strength was found sufficient to prevent the growth of fungi, up to the present date a number of methods have been practised which may be summarized as follows: Sterilization by heat, by the addition of an antiseptic, by the combination of these two methods, and by the chemical synthesis of alkaloids with antiseptic acids.

Particular attention has been directed to cocaine. Sattler, for example, was accustomed to prepare a solution of this alkaloid by adding to it a 1 : 5000 solution of bichloride of mercury, which later was changed to a 1 : 10,000 solution upon the strength of some investigations made by Herrnheiser. The same method of preparing cocaine is extensively used by many surgeons.

Eversbusch boiled the solution preparatory to an operation, a procedure which sterilizes the fluid itself, but does not prevent the entrance of bacteria from the surrounding air.

Hirschberg, with characteristic thoroughness, first sterilized his solution in the ordinary manner in a sterilizing apparatus, and then added to it sublimate 1 : 5000.

[1] Berlin klin. Wochenschr. 1888, No. 35; quoted by Franke, loc. cit.

Finally, an attempt to secure an antiseptic drug has been made by combining eserine and cocaine with salicylic acid in the form of salicylate of eserine and salicylate of cocaine, a pharmaceutical experiment at one time indorsed by Galezowski and Petit.

As there seemed objections to some of the procedures, and uncertainties in regard to others, Franke has reviewed the whole subject, endeavoring to discover the simplest and safest method of sterilizing eye lotions, and has devoted himself to the sulphates of atropine and eserine and the hydrochlorate of cocaine, the first in 1 per cent. and the second in 2 per cent. solution.

The most important conclusions given by the author at the close of his paper are here summarized: Chemical disinfection of " eye drops " is a method in general to be preferred to a sterilization by heat, because by the latter procedure the lotions are not protected from the micrococci which may fall into them from the surrounding air. The drugs which may be employed for this purpose are sublimate 1 : 5000 and 1 : 10,000, oxycyanide of mercury 1 : 1000 to 1 : 1500, resorcin 1 per cent, carbolic acid $\frac{1}{2}$ per cent., boric acid 4 per cent. with 1 per cent. of carbolic acid, Panas's fluid, and thymol in the form of thymol water, or chloroform water. The last two substances have the disadvantage of producing smarting and burning of the conjunctiva. In their behavior toward the staphylococcus pyogenes flavus and Michel's trachoma coccus, sublimate 1 : 1000, oxycyanide of mercury 1 : 1000, and thymol water, surpass the others.

It is not possible, however, for ophthalmic purposes to use these lotions in a strength sufficient to obtain an absolutely antiseptic action, but in general it is safe to add a portion of sublimate lotion (1 : 10,000) to atropine and cocaine solutions, in order to render them aseptic for a space of from one-half to one hour.

Sterilization alone, of atropine and cocaine solutions, when they are used in operative work, without the addition of an antiseptic, is not nearly so satisfactory as a combination of the two methods.

The addition of a 1 : 10,000 sublimate lotion to an atropine solution prevents atropine conjunctivitis.

In addition to these precautions, it is recommended that the bottles and pipettes be sterilized by boiling and mechanical cleaning. In short, two methods of sterilization are practicable, namely, the addition of a chemical substance and the use of heat, and even Franke does not feel quite safe unless he employs both.

Pergens,[1] after reviewing the various methods of sterilization, and pointing out the advantages and disadvantages of each, suggests that hypodermic tablets, which have been carefully manufactured and well dosed, shall be prepared. These are dissolved in sterilized water and poured into bottles of the capacity of three to five grammes. Each patient is treated with a different tube, which is then plunged in the sublimate solution, where it remains for several hours. These pipettes are washed with water and sterilized with steam, because he believes that they are the principal source of infection.

Heat alone, however, is entirely sufficient, if proper precautions are taken that the surrounding bacteria shall not fall into the fluid, and if a suitable method be employed for heating the fluids. To this end Stroschein[2] has devised a new bottle, or rather a combination of flask and pipette. He has had blown-glass bottles constructed with droppers which may be directly exposed to the flame, thus rapidly sterilizing the bottle and its contents. The pipette has two conical ends, and is introduced into the neck of the bottle point upward, the rubber head being removed before the reversal. If the collyria are to be boiled the small tube gives free vent to the steam, which, passing through it, at the same time sterilizes it. Experiments have shown the inventor that the collyria need to be boiled only three or four minutes to render them perfectly sterile, and for the point of the tube, which is directed upward during the ebullition, likewise to become sterile. The loss of water which is produced by a

[1] Annales d'Oculistique, December, 1891.

[2] Archiv. f. Ophthalmologie, Bd. xxxviii, Abth. 2, p. 155.

boiling of three or four minutes is about one cubic centimetre, hence, if concentration of the solution is to be avoided, fifteen drops of water should be added before the boiling.

A number of so-called antiseptic droppers have been devised, one well-known pattern consisting of a combination of the dropper and glass stopper in a single piece of glass. All of these devices are ingenious, but do not secure the introduction into the eye of an aseptic fluid, because, as has been proved over and over again, the water in which the drug is dissolved may not be clean, and solutions of the alkaloids, with the possible exception of eserine, even when freshly prepared, will usually yield a growth of fungi and various micrococci in culture media.

Although the question of infection and sterilization of eye lotions has so frequently and so thoroughly been investigated, we desire to present the results thus far obtained in a research the object of which was to ascertain, purely for our own satisfaction, the condition of the fluids, pipettes, and bottles contained in a case which had been much used in the treatment of various ocular disorders, although never in operative work, and with which no special precautions had been taken. The examinations are not complete; but they have developed some points of interest, and these may be briefly stated.

The following fluids were taken from the bottles and carefully placed in sterilized flasks and suitably corked: Boric acid, 15 grains to the ounce of distilled water; bichloride of mercury 1 : 10,000; nitrate of silver 1, 5 and 10 grains to the ounce respectively; sulphate of atropine, 4 grains to the ounce; hydrobromate of homatropine, 8 grains to the ounce; hydrochlorate of cocaine, four per cent.; sulphate of eserine, 1 grain to the ounce; pyoktanin 1 : 1000; and Gruebler's fluorescine, two per cent.

The pipettes which had been used in these fluids, and with two exceptions in them only, as each one is attached to its own bottle by a special arrangement, were also placed in carefully sterilized flasks and examined.

The droppers were tested in the following manner: Thor-

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oughly sterilized distilled water was drawn up into each pipette until the latter was three-quarters full, and then forced out by the rubber bulb and allowed to drop into potato tubes, peptonized beef broth, and agar-agar. The tubes were then placed in the incubator and examined at the end of forty-eight hours.

The cocaine and eserine droppers treated in this way gave, in forty-eight hours, growths on all three of the culture media, which upon examination proved to be a mixed culture of long and short bacilli and micrococci.

After two weeks a growth was noticed on the potato culture from the fluorescine pipette; but it is not certain that this was not the result of a contamination.

Other droppers tested in exactly the same way, namely, those from the atropine, homatropine, boric acid, and pyoktanin solutions and an unused dropper, yielded no growth whatever.

The lotions themselves were treated by transferring to the culture media—potato, peptonized beef broth, agar-agar—by means of a platinum loop, drops of the liquid to be examined. The cocaine, boric acid, atropine, and homatropine lotions developed growths on potato, peptonized beef broth, and agar-agar. Drop inoculations from the fluorescine were negative, but when one-half drachm was used an abundant fungus growth appeared. The other solutions—namely, eserine, nitrate of silver, pyoktanin, and bichloride of mercury—developed nothing at all.

The pathogenic effects of the cultures so far obtained, though not pure, were next tried. The agar-agar cultures were the source of the material for injection, and an emulsion of the surface growth and the condensation water of the culture was used, one-tenth of a cubic centimetre for each injection. The injections were made by means of a hypodermic syringe into the anterior chamber of a rabbit's eye, care being taken not to injure the lens. The cultures from which injections were made were from the boric acid lotion, cocaine, fluorescine (dropper), homatropine and eserine (dropper).

The eserine growth (pipette) caused in the eye of the rabbit a slight inflammation of the iris which disappeared in two days.

The growth from homatropine produced a mild iritis which lasted for four days.

The culture from the fluorescine dropper provoked a slight iritis associated with a moderate keratitis which disappeared spontaneously in eight days.

The cultures from the boric acid and the cocaine, the first derived from the solution and the second from the pipette, produced a violent hypopyon-keratitis and purulent iritis.

Rabbits were then taken and the corneæ abraded with a sterilized needle. The boric acid and cocaine cultures were dropped on eyes thus prepared, and rubbed over the abraded surfaces. The result was a slight ciliary injection in the region of the abrasion and a moderate haze in the cornea surrounding it; this condition lasted for two or three days, and was more pronounced than the inflammation produced by a simple abrasion of the cornea without the application of the germs.

In order to control the injections in the anterior chamber, the eserine solution, which had not produced a growth upon the culture media, was injected, without results; hence the hypopyon-keratitis developed was not the effect of the fluid, but of the bacilli and fungi which it contained. Further control experiments with negative results were made by the injection of sterile culture liquids.

The cultures from the boric acid and cocaine show under the microscope long and short bacilli and micrococci; proteus vulgaris and bacterium termo (Vignal) have been isolated. The bacilli have the general appearance of the ordinary water bacilli. Sufficient time has not yet elapsed to separate them in a pure culture. The homatropine growth and the one from the eserine dropper showed short fat bacilli. The atropine and fluorescine (dropper) cultures are the ordinary fungi.

The experiments in detail, with the daily appearance of the eyes, follow:

November 30, 1892. Gray rabbit No. 1; weight four pounds. Received $\frac{1}{10}$ c.c. of the culture obtained from the eserine dropper.

December 1. The eye was very slightly injected and iris hyperæmic.

2*d*. Eye entirely well.

November 30, 1892. Gray rabbit No. 2; weight four and one-fourth pounds. Received an injection in the left eye of $\frac{1}{10}$ c.c. culture from the fluorescine dropper.

December 1. Centre of the cornea cloudy.

2*d*. Cornea still clouded; the bloodvessels of the iris decidedly injected.

3*d*. Still a slight inflammation of the iris; apparently a little lymph in pupillary space, but the cornea no longer cloudy.

5*th*. Iris still slightly inflamed; lymph had almost entirely disappeared.

6*th*. Slight iritis.

7*th*. Slight iritis, but better.

9*th*. Still a very slight inflammation.

13*th*. Eye entirely well.

November 30, 1892. Rabbit No. 3; weight four pounds. Received $\frac{1}{10}$ c.c. from culture growth of homatropine solution.

December 1. Iris a little inflamed and a slight cloudiness in pupillary space.

2*d*. Eye still inflamed.

3*d*. Inflammation subsiding.

5*th*. Eye almost normal.

7*th*. Slight iritis.

9*th*. Slight iritis.

14*th*. Eye perfectly well.

November 30, 1892. Brown rabbit No. 4; weight four pounds. Received $\frac{1}{10}$ c.c. of culture from cocaine dropper.

December 1. Cornea very cloudy and inflamed.

2*d*. Eye swollen, inflamed, suppurating, and hypopyon.

3*d*. Extensive iritis.

7*th*. Extensive purulent iritis.

9*th*. Eye about the same as on December 7th, except that the bloodvessels in the cornea had become very much more prominent.

14*th*. Eye in the same condition as on December 9th.

February 7, 1893. Eye removed. There had been practically no change in its appearance since December 14th.

November 30, 1892. Rabbit No. 5; weight four and one-half pounds. Received an injection of $\frac{1}{10}$ c.c. of culture from the boric acid solution.

December 1. Cornea clouded.

2*d*. Still slightly clouded, and the iris hyperæmic.

3*d*. Extensive iritis and keratitis.

5th. Eye apparently better.

6th. Relapse; severe iritis and keratitis.

7th. Still severe kerato-iritis.

9th. Same as on December 6th.

13th. Entire cornea infiltrated; severe hypopyon-keratitis.

February 7, 1893. Eye removed for section; apparently the same condition as on December 13th.

December 13, 1892. Gray rabbit No. 6; weight four pounds. Received $\frac{1}{10}$ c.c. in left eye, of culture obtained from cocaine solution. This solution when first tested in October had shown no growth, but when cultures were made again in December, a decided growth was developed upon agar, consisting of long and short bacilli.

February 7. When the eye was removed its appearance was almost identical with that of the boric acid and other cocaine eye, viz., suppurative kerato-iritis.

January 7, 1893. Rabbit No. 7; weight three and one-half pounds. Cornea of right eye was scraped with a sterile knife blade, and some of the boric acid culture dropped on the abraded spot and rubbed over it.

9th. A slight injection of the bloodvessels in ciliary region just above the point where surface was abraded. A small cloudy spot, the size of a pin-head, formed about the centre of the line of abrasion.

12th. Eye normal.

January 7, 1893. Maltese rabbit No. 8; weight three pounds. Surface of cornea abraded and a few drops of the culture from cocaine solution rubbed in.

8th. Slight reddening in ciliary region and cloudiness in cornea.

12th. Faintest cloudiness in cornea; otherwise quite well.

February 9. Eye normal.

January 7, 1893. Black Angora rabbit No. 9; weight four pounds. Surface of cornea scraped and a few drops of culture from cocaine dropper rubbed in.

9th. Injection of bloodvessels in ciliary regions above the point where the surface was scraped.

12th. Inflammation has subsided; a very slight cloudiness above where the surface was abraded.

This experiment of scraping the cornea was repeated again on January 21st, with exactly the same results as those recorded. Simply scraping the cornea produced a slight injection of the bloodvessels in the ciliary regions; not so much, however, as when the cultures were subsequently applied.

A summary of the results, arranged in a tabular manner, follows:

PIPETTES.

Unused pipette.	No growth.		
Cocaine pipette.	Growth in potato, agar-agar, and beef broth.	Same germs as those found in cocaine solution; inoculation caused purulent irido-choroiditis.	Rubbing abraded cornea with culture produced moderate ciliary injection and slight clouding of cornea.
Fluorescine pipette.	Growth on potato.	Inoculation caused slight iritis.	
Atropine pipette.	No growth.	Pipette had been frequently cleansed with sublimate lotion.	
Pyoktanin pipette.	No growth.		
Homatropine pipette.	No growth.		
Eserine pipette.	Growth on all three culture media.	Slight iritis caused by inoculation which speedily disappeared.	
Boric acid pipette.	No growth.	This pipette had often been used with a sublimate lotion.	

FLUIDS.

Boric acid lotion.	Active growth on all three culture media.	Inoculation into anterior chamber produced purulent irido-chor-oiditis.	Rubbing abraded cornea with culture produced moderate ciliary injection and slight clouding of cornea.
Atropine lotion.	No growth at first; one month later active growth.	The growth, a fungus, was not used in inoculation, as it was of same nature as that obtained from the fluorescine dropper.	
Homatropine lotion.	Growth on all three culture media.	Inoculation produced slight iritis which disappeared in fifteen days.	
Cocaine lotion.	No growth at first; two months later active growth on all three culture media.	Inoculation produced purulent irido-chor oiditis.	Rubbing abraded cornea with culture produced moderate ciliary injection and slight clouding of cornea.
Fluorescine lotion.	Abundant fungus growth.	Same growth as from pipette not used in inoculation.	
Bichloride lotion.	No growth.		
Nitrate of silver lotion.	No growth.		

The two eyes which had suffered most severely from the injections of the cultures, viz., from the cocaine and the boric acid, were removed and submitted to microscopic examination. As the lesions are practically the same in each, one description will suffice. The iris is infiltrated with leucocytes, and there is a large layer of pus on its posterior surface, in some specimens practically filling the posterior chamber. The ciliary body and choroid are densely infiltrated with darkly stained corpuscles, the

retina is detached and infiltrated, and the papilla inflamed. In brief, there is purulent irido-choroiditis with secondary involvement of the retina and optic nerve.

We are indebted to Dr. William M. Gray for preparing these sections.

These experiments, in general and as far as they go, confirm the oft-repeated observation that solutions of the alkaloids contain mould-fungi, *e. g.*, aspergillus glaucus, saprophytic bacteria, and sarcina lutea. They also confirm the observations which have been recorded, that these solutions, when they have been some time in use, and when proper precautions are not taken to sterilize them, may be contaminated with pathogenic micro-organisms which are capable of producing by inoculation into a normal eye a purulent irido-choroiditis.

Referring for a moment to the particular results, it is interesting that one of the two most virulent cultures was obtained from the boric acid lotion, a culture which evidently contained pathogenic germs. The source of these micro-organisms may, perhaps, be explained by Franke's idea that they are introduced into the fluid by reason of the pipette coming in contact with the cilia or conjunctiva of an eye which contains pathogenic bacteria, and it is likely that this lotion was frequently used for irrigating inflamed conjunctivæ. It serves as an illustration of the well-known fact that boric acid is an antiseptic substance of very indifferent power, the minimum degree of concentration in a watery solution in which it is reliable, according to Miquel, being 1 : 13. In the solution with which we experimented the concentration was 1:32.

It is further interesting to note that the solution of eserine yielded no growth upon the culture medium, and that when it was injected into the anterior chamber it proved to be innocuous. This is somewhat in accord with some of Franke's observations, who found in a few inoculations from freshly prepared eserine solutions, and also from those which were several days old, that the tubes remained free from growths. The eserine pipette yielded a growth of long and short bacilli and micrococci, consequently its contamination must have come from outside sources which did not gain entrance into the fluid in the bottle.

If it be assumed that the culture from the fluorescine dropper was not due to a contamination (it did not appear until two weeks after the potato tube was inoculated), it is worthy of remark that it produced a slight iritis when injected into the anterior chamber. Fluorescine solution has been much used in recent times to demonstrate the extent of corneal ulcers, to locate small foreign bodies, and to expose, by virtue of its power to color green those portions of the cornea which are deprived of superficial epithelium, any loss of substance in this membrane, whether caused by injury or by disease. Hence there is a possibility that by this means micro-organisms might be conveyed to the eye and convert a simple abrasion into an unhealthy ulcer. In our experiment the fluid itself was at first apparently sterile, but subsequently inoculations gave an abundant fungus growth of the same character as that obtained from the dropper.

That mere contact of the cultures with an abraded cornea is unlikely to produce serious inflammatory reaction or purulent infection is evident from our last four experiments, in which the infecting agent was rubbed into a wound of the cornea made with a sterilized knife. Ciliary injection and slight corneal infiltration occurred, which subsided in a few days without treatment, but destructive keratitis did not supervene in any case, although the same cultures injected into the anterior chamber caused purulent irido-choroiditis.

Hence we have a reason for the comparative rarity of untoward results from non-sterile solutions that are so frequently (and almost of necessity) used in eyes manifesting all manner of lesions, but in which the anterior chamber is not opened.

They also furnish additional evidence that unclean solutions which find their way into the anterior chamber, *e.g.*, after an operation, are capable of speedily originating a destructive inflammation of the uveal tract, terminating in panophthalmitis, and emphasize the importance of securing perfect sterilization of *any* lotion, especially of cocaine, which is to be used in a case requiring corneal section; showing also that boric acid solution which is not freshly prepared (gr. xv to f℥j) may be the medium of the most virulent contamination.